

WHAT IS CLAIMED:

1. A method of preparing a highly phosphorylated acid β -glucocerebrosidase comprising:
- (a) contacting an acid β -glucocerebrosidase with an isolated GlcNAc phosphotransferase to produce a modified acid β -glucocerebrosidase; and
- (b) contacting said modified acid β -glucocerebrosidase with an isolated phosphodiester α -GlcNAcase.
2. The method of Claim 1, further comprising purifying said highly phosphorylated acid β -glucocerebrosidase after said contacting with the isolated phosphodiester α -GlcNAcase.
3. The method of Claim 1, further comprising purifying said modified acid β -glucocerebrosidase prior to said contacting with the isolated phosphodiester α -GlcNAcase.
4. The method of Claim 1, wherein said isolated GlcNAc phosphotransferase comprises an α subunit and β subunit.
5. The method of Claim 4, wherein the GlcNAc phosphotranferase comprises the amino acid of SEQ ID NO:2. p10
6. The method of Claim 1, wherein the GlcNAc phosphotranferase comprises SEQ ID NO:4 and SEQ ID NO:5.

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7. The method of Claim 1, wherein the GlcNAc phosphotranferase is encoded by a nucleotide sequence comprising SEQ ID NO:1, or a sequence that hybridizes under stringent conditions to SEQ ID NO:1.
- 5 8. The method of Claim 1, The method of Claim 1, wherein the GlcNAc phosphotranferase is encoded by a nucleotide sequence comprising SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to SEQ ID NO:3.
- 10 9. The method of Claim 1, wherein the phosphodiester α -GlcNAcase comprises SEQ ID NO:17 or a sequence that hybridizes under stringent conditions to SEQ ID NO:17.
- 15 10. The method of Claim 1, wherein the acid β -glucocerebrosidase comprises the amino acid sequence of SEQ ID NO:25 or SEQ ID NO:26.
11. The method of Claim 1, wherein the acid- β -glucocerebrosidase comprises the amino acid sequence of SEQ ID NO:26.
- 20 12. A highly phosphorylated acid β -glucocerebrosidase obtained by the method of Claim 1.
13. A pharmaceutical composition comprising the highly phosphorylated acid β -glucocerebrosidase of Claim 12 and a pharmaceutically acceptable carrier.
- 25 14. A method of treating a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid

beta-glucocerebrosidase of Claim 12 in an amount sufficient to treat said disease.

15. The method of Claim 14, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.

16. A method of treating a bone tissue of a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 12 in an amount sufficient to treat said disease.

17. The method of Claim 16, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.

18. A method of treating a lung tissue of a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 12 in an amount sufficient to treat said disease.

19. The method of Claim 18, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.

20. A method of producing a highly phosphorylated acid β -glucocerebrosidase comprising:

(a) culturing transfected cells comprising a recombinant polynucleotide which encodes a recombinant acid β -glucocerebrosidase in the presence of at least one α 1,2-mannosidase inhibitor;

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- (b) recovering a high mannose recombinant acid β -glucocerebrosidase from said transfected cell;
- (c) contacting said high mannose recombinant acid β -glucocerebrosidase with an isolated GlcNAc phosphotransferase to produce a modified acid β -glucocerebrosidase; and
- (d) contacting said modified acid β -glucocerebrosidase with an isolated phosphodiester α -GlcNAcase.

21. The method of Claim 20, wherein said at least one 1,2-mannosidase inhibitor is selected from the group consisting of deoxymannojirimycin, kifunensine, D-Mannonolactam amidrazone, and N-butyl-deoxymannojirimycin.

22. The method of Claim 21, wherein the 1,2-mannosidase inhibitor is kifunensine.

23. The method of Claim 21, wherein the 1,2 mannosidase inhibitor is deoxymannojirimycin.

24. The method of Claim 21, wherein the at least one 1,2 mannosidase inhibitor is deoxymannojirimycin and kifunensine.

25. The method of Claim 20, further comprising purifying said modified acid β -glucocerebrosidase prior to said contacting with the isolated phosphodiester α -GlcNAcase.

26. The method of Claim 20, wherein said isolated GlcNAc phosphotransferase comprises an α subunit and β subunit.

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27. The method of Claim 26, wherein the GlcNAc phosphotransferase comprises the amino acid SEQ ID NO:2.
- 5 28. The method of Claim 20, wherein the GlcNAc phosphotransferase comprises SEQ ID NO:4 and SEQ ID NO:5.
29. The method of Claim 20, wherein the GlcNAc phosphotransferase is encoded by a nucleotide sequence comprising SEQ ID NO:1, or a sequence that hybridizes under stringent conditions to SEQ ID NO:1.
- 10 30. The method of Claim 20, The method of Claim 1, wherein the GlcNAc phosphotransferase is encoded by a nucleotide sequence comprising SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to SEQ ID NO:3.
- 15 31. The method of Claim 20, wherein the phosphodiester α -GlcNAcase comprises SEQ ID NO:17 or a sequence that hybridizes under stringent conditions to SEQ ID NO:17.
- 20 32. The method of Claim 20, wherein the acid β -glucocerebrosidase comprises the amino acid sequence of SEQ ID NO:25 or SEQ ID NO:26.
- 25 33. The method of Claim 20, wherein the acid- β -glucocerebrosidase comprises the amino acid sequence of SEQ ID NO:26.

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34. A highly phosphorylated acid β -glucocerebrosidase obtained by the method of Claim 20.
- 5 35. A pharmaceutical composition comprising the highly phosphorylated acid β -glucocerebrosidase of Claim 34 and a pharmaceutically acceptable carrier.
- 10 36. A method of treating a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 34 in an amount sufficient to treat said disease.
- 15 37. The method of Claim 36, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.
- 20 38. A method of treating a bone tissue of a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 34 in an amount sufficient to treat said disease.
- 25 39. The method of Claim 38, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.
40. A method of treating a lung tissue of a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 34 in an amount sufficient to treat said disease.

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41. The method of Claim 40, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.
- 5 42. A highly phosphorylated acid β -glucocerebrosidase, which is encoded by the nucleotide sequence of SEQ ID NO:24 of a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:24.
- 10 43. The highly phosphorylated acid β -glucocerebrosidase of Claim 28, which comprises the amino acid sequence of SEQ ID NO:25 or SEQ ID-NO:26.
44. The highly phosphorylated acid β -glucocerebrosidase of Claim 43, which comprises the amino acid sequence of SEQ ID NO:26.
- 15 45. A pharmaceutical composition comprising the highly phosphorylated acid β -glucocerebrosidase of Claim 42 and a pharmaceutically acceptable carrier.
- 20 46. A method of treating a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 42 in an amount sufficient to treat said disease.
- 25 47. The method of Claim 46, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.
48. A method of treating a bone tissue of a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly

phosphorylated acid beta-glucocerebrosidase of Claim 42 in an amount sufficient to treat said disease.

49. The method of Claim 48, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.

50. A method of treating a lung tissue of a patient suffering from Gaucher's disease, comprising administering to the patient in need thereof the highly phosphorylated acid beta-glucocerebrosidase of Claim 42 in an amount sufficient to treat said disease.

51. The method of Claim 50, further comprising administering acid β -glucocerebrosidase which is not highly phosphorylated.

52. A method of preparing a highly phosphorylated acid β -glucocerebrosidase comprising:

- (i) a step for transferring a N-acetylglucosamine-1-phosphate from UDP-GlcNAc to an acid β -glucocerebrosidase;
- (ii) a step for removing an N-acetylglucosamine from said acid β -glucocerebrosidase.

53. A highly phosphorylated acid β -glucocerebrosidase obtained by the method of Claim 52.

54. The highly phosphorylated acid β -glucocerebrosidase of Claim 53, which comprises the amino acid sequence of SEQ ID NO:26.

55. A pharmaceutical composition comprising the highly phosphorylated acid β -glucocerebrosidase of Claim 53 and a pharmaceutically acceptable carrier.
56. A method of treating a patient suffering from Gaucher's disease, comprising
5 administering to the patient in need thereof the highly phosphorylated acid
beta-glucocerebrosidase of Claim 53 in an amount sufficient to treat said
disease.
57. The method of Claim 56, further comprising administering acid β -
10 glucocerebrosidase which is not highly phosphorylated.
58. A method of treating a bone tissue of a patient suffering from Gaucher's
disease, comprising administering to the patient in need thereof the highly
phosphorylated acid beta-glucocerebrosidase of Claim 53 in an amount
15 sufficient to treat said disease.
59. The method of Claim 58, further comprising administering acid β -
glucocerebrosidase which is not highly phosphorylated.
60. A method of treating a lung tissue of a patient suffering from Gaucher's
20 disease, comprising administering to the patient in need thereof the highly
phosphorylated acid beta-glucocerebrosidase of Claim 53 in an amount
sufficient to treat said disease.
61. The method of Claim 60, further comprising administering acid β -
25 glucocerebrosidase which is not highly phosphorylated.